HIV-1 RNA Rectal Shedding Is Reduced in Men With Low Plasma HIV-1 RNA Viral Loads and Is Not Enhanced by Sexually Transmitted Bacterial Infections of the Rectum

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Background. Among human immunodeficiency virus (HIV)–infected men who have sex with men (MSM) taking combination antiretroviral therapy (cART), the impact of rectal sexually transmitted infections (STIs) on rectal HIV-1 shedding is unknown.

Methods. Human immunodeficiency virus type 1 (HIV-1) RNA was quantified from rectal swabs collected for *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT) screening of HIV-1-infected MSM. Correlations of STIs with rectal viral load were explored using multinomial regression modeling. HIV-1 coreceptor tropism was predicted from sequencing in a subset of men.

Results. Thirty-one (39%) of 80 men (59 prescribed combination antiretroviral therapy [cART]) had HIV detected in 38 (42%) of 91 rectal swabs. Rectal HIV detection was associated with plasma virus loads above 3.15 $\log_{10}$ copies/mL (95% confidence limit [CL] 2.73, 3.55) and paired rectal viral loads and plasma viral loads were correlated (Kendall's tau [τ] 0.68, Spearman rho [P] = .77). Rectal STIs and abnormal anal cytology were not associated with rectal viral load. HIV coreceptor distribution was very similar between the plasma and rectum in 3 of 4 men.

Conclusions. Plasma and rectal viral load were correlated, and rectal STIs did not increase the likelihood of detecting HIV in the rectal secretions in MSM, including those with low or undetectable plasma viral load. Suppressing plasma viral load is likely to reduce risk of HIV transmission to insertive partners.

Over half (53%) of new human immunodeficiency virus (HIV) infection diagnoses in the United States from 2001–2006 were among men who have sex with men (MSM), accounting for almost 100 000 new infections [1]. During this time period, both the incidence and prevalence of HIV among MSM increased significantly after both trends had steadily declined during the 1990s [2–4]. Anal intercourse, principally among MSM, remains the primary mode of HIV transmission in the United States, and insertive anal intercourse among MSM accounts for 28% of new infections [5], making rectal secretions an important potential source of HIV transmission. MSM, including HIV-infected MSM, also experience high incidences of other sexually transmitted infections (STIs), notably rectal and oral *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT). Although urethral STIs increase HIV shedding in semen, the effect of rectal STIs on HIV shedding into rectal secretions, especially among MSM with suppressed plasma HIV viral loads, is largely undescribed [6–8]. No studies have explored the influence of rectal GC or CT infection on HIV shed in rectal secretions.

The vast majority of transmitted HIV is CCR5-tropic for reasons that are incompletely understood [9–11]. There are few data on the molecular characteristics of HIV shed in the rectum, as most previous studies have

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focused on rectal biopsies or feces [12–15]. Further exploration into the molecular characteristics of HIV in rectal secretions is therefore warranted in light of the potential significant role they play in HIV transmission and as a target for biomedical prevention interventions.

One barrier to this research is the difficulty in obtaining clinical samples, as previous methods employed anoscopy [16–19]. Therefore, we sought to determine the applicability of rectal swabs collected without anoscopy for GC and CT screening for measuring HIV shedding in rectal secretions. In addition, we sought to determine the effect of rectal GC or CT infection on HIV rectal viral load in a contemporary US sample of HIV-infected MSM with access to combination antiretroviral therapy (cART). Finally, to enhance our understanding of the molecular factors that contribute to HIV transmission from rectal secretions, we compared HIV coreceptor (CCR5 vs CXCR4) usage between viruses found in plasma and rectal secretions among a subset of men in the cohort.

METHODS

The SUN Study

The Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy (the "SUN" study) is a prospective observational cohort study of 700 HIV-infected participants, of whom 425 are MSM, enrolled from 7 clinics in 4 US cities: Denver, CO; Minneapolis, MN; Providence, RI; and St. Louis, MO. Enrollment took place between March 2004 and June 2006. Informed consent was obtained from all study participants, and the study was approved and has been renewed annually by the institutional review boards of all participating institutions and the Centers for Disease Control and Prevention (CDC). The cohort and detailed study methodology have been described elsewhere [20]. Briefly, at baseline and every 6 months thereafter, data were collected through physical examination, audio computer-assisted self-interview, noninvasive imaging, laboratory examination, and medical chart abstraction. Demographic information as well as clinical information was collected, including $CD4^+$ T-cell counts, plasma viral load, herpes simplex virus 1 and 2 serostatus (HSV-1 and HSV-2), annual anal cytology and anal human papillomavirus (HPV) DNA testing, and medication histories. In addition, at study enrollment and every 6 months thereafter, participants were screened for genitourinary, oropharyngeal, and rectal GC and CT infections (see below). For this substudy, we included samples from all MSM diagnosed with rectal GC or CT infection from 2006 through 2009 as well as a convenience sample of swabs from MSM across the plasma viral load spectrum (ie, 60 swabs from men with varying levels of plasma viral load were analyzed) without rectal GC or CT infection. No samples from the Providence site were included in this analysis, as these were not stored at CDC.

Rectal Swab Collection and Testing

Clinicians collected rectal specimens for STD testing by blindly inserting the Dacron-tipped swabs approximately 3–5 cm into the rectum. The swabs were then rotated against the rectal wall several times. Swabs grossly contaminated with fecal material were discarded and the procedure repeated. Swabs were placed into Gen-Probe APTIMA media (San Diego, CA) and shipped overnight to CDC. Swabs were first tested for GC and CT by nucleic acid amplification testing (NAAT) with the APTIMA Combo 2 Assay and then stored at –20°C. The CDC internally validated the APTIMA Combo 2 Assay for rectal specimens for this clinical use in accordance with the Clinical Laboratory Improvement Act of 1988.

Rectal Swab HIV RNA Extraction and Quantification

HIV-1 RNA was quantified from 1-mL aliquots of Gen-Probe APTIMA media. The media were not assayed for the presence of semen prior to RNA extraction. The aliquots were centrifuged at 1000 × g for 2 minutes to remove fecal particulates and cellular material. The resulting supernatants were passed through a 0.45-μm-pore-size filter that was prewetted with cell culture medium and then centrifuged (43 000 × g, 30 minutes) to pellet viral particles. The supernatants were removed and nucleic acids in the pellets were extracted using the Omega Bio-Tek E.Z.N.A. Total RNA kit (Doraville, GA). HIV-1 RNA was amplified by reverse transcription–polymerase chain reaction (RT-PCR) and quantified from 50 μL of the extract using the Roche Amplicor HIV-1 Monitor Ultrasensitive Assay (Branchburg, NJ). HIV-1–negative and low-copy-number HIV-1 RNA controls were included in each PCR run. Using the above extraction and amplification procedures, virus spiking experiments showed a highly reproducible sensitivity for quantifying 145 HIV-1 RNA copies/swab. In addition, we were able to accurately detect HIV-1 RNA below the assay's quantification limit when the RT-PCR signal was >2-fold above negative control values; these detectable but less-than-quantifiable signals were placed in category 2 for analyses (see below).

HIV Coreceptor Prediction

In an exploratory analysis, HIV coreceptor usage (CCR5 vs CXCR4) in HIV-1 RNA quasispecies of blood and rectal secretions was predicted from 4 men using sequenced clones of the C2V4 region of Env_{gp120} as previously described [21, 22]. Twenty colonies each from plasma and rectal secretions were selected and sequenced. The plasmid inserts were sequenced using the Beckman Coulter CEQ (Brea, CA). Sequences were trimmed to the V3 loop with the Sequencher 4.8 program (Gene Codes Corp., Ann Arbor, MI), aligned, and translated using the Los Alamos HIV sequence database tools (available at: http://www.hiv.lanl.gov/content/sequence/CodonAlign/codonalign.html). Coreceptor tropism was determined using the PSSM program (available at: http://indra.mullins.microbiol.washington.

edu/webpssm/). Phylogenetic analyses showed strong intrasubject clustering among rectal swab and plasma quasispecies, with no evidence of contamination or intersubject mixing of clones.

Statistical Analyses

Descriptive statistics for demographic and clinical variables were tabulated as medians or percentages. Values for clinical or laboratory variables (eg, plasma viral load, $CD4^+$ T-cell count, anal HPV and cytology results) were assigned according to the result closest in time to the date of rectal swab collection for STD screening. Square-root transformation of $CD4^+$ T-cell count was used to homogenize variability. Rectal viral load and plasma viral load were categorized by increasing logarithm base-10 increments (ie, undetectable = category 1, 1.86–3.0 $\log_{10}$ = category 2, >3.0–4.0 $\log_{10}$ = category 3, >4.0–5.0 $\log_{10}$ = category 4, >5.0 $\log_{10}$ = category 5). Correlations were assessed by calculating Kendall tau (τ) rank correlation and Spearman rho (ρ) rank correlation coefficients for the relationship between rectal viral load and plasma viral load.

To determine clinical correlates of rectal viral load, we performed multinomial regression on categories of rectal viral load to account for the large proportion of viral load measurements below the limit of detection [23]. Robust variances were calculated to account for within-subject correlation for those men in the study with multiple observations. A score test was implemented to test the assumption of proportional odds. To estimate a threshold of plasma viral load that distinguished detectable from undetectable rectal viral load, we performed logistic regression of the binomial outcome of detectable or undetectable rectal viral load on detectable $\log_{10}$-transformed plasma viral load as the predictor variable.

RESULTS

We assayed 93 swabs from 81 MSM for HIV rectal viral load. Of these, the nucleic acid extracts from 2 swabs inhibited the RT-PCR assay on 2 separate attempts and were excluded from the analysis. One of the inhibited swab samples was from a man who had another rectal swab analyzed from a separate clinic visit; thus, data from 91 paired plasma and rectal specimens from 80 MSM were used in our analysis. Demographic and clinical characteristics of the 80 MSM included are listed in Table 1. Seventy-four percent of men were prescribed cART. Their median $CD4^+$ T-cell count at the time of first swab collection was 467 cells/μL, and 63% of the participants had plasma viral loads <3.0 $\log_{10}$ copies/mL. Fifty-four (67%) men were HSV-2 seropositive, 76 (95%) had anal HPV infection (ie, detectable HPV DNA), and 33 (41%) had abnormal anal cytology, defined as the presence of atypical squamous cells of undetermined significance or greater per the Bethesda system. Thirty-one (34%) of 91 rectal samples from 31 (39%) men in our analysis were positive for GC or CT by NAAT testing; 19 (21%) samples were CT positive, 9 (10%) were GC positive, and 3 (3%) were positive for CT and GC. Two men diagnosed with rectal GC or CT infection reported symptoms of proctitis at the time of sampling; the remaining 94% of rectal infections were asymptomatic.

Rectal HIV RNA was detected from 30 (38%) men overall and 38 (42%) of 91 rectal swabs. Rectal viral load and plasma viral load were highly correlated (τ = 0.68, 95% confidence limit [CL] 0.59, 0.77; ρ = 0.76, 95% CL 0.66, 0.86; Figure 1). Correlation between plasma viral load and rectal viral load, as estimated by τ, for specimens from MSM with and without rectal GC or CT was 0.73 (95% CL 0.53, 0.92) and 0.66 (95% CL 0.55, 0.78), respectively. Although these correlation coefficients did not differ significantly, we found significant differences in the strength of the association between rectal viral load and plasma viral load according to whether rectal GC or CT infection was present or absent as determined by inclusion of a 2-way interaction term in a multivariable multinomial regression model (P = .01). This result supports a stronger correlation between rectal viral load and plasma viral load for MSM with rectal GC or CT infection.

HIV was considerably less likely to be detected in rectal swabs from men with plasma viral load <3.0 $\log_{10}$ copies/mL than ≥3.0 $\log_{10}$ copies/mL (9% vs 87%; P <.001), and the presence of rectal GC or CT infection did not alter the likelihood of detecting HIV in rectal swabs at lower (<3.0 $\log_{10}$ copies/mL, P = .39) or higher plasma viral load (≥3.0 $\log_{10}$ copies/mL, P = .99). In addition, based on the predicted value from a logistic regression model, the plasma viral load threshold above which rectal viral load is expected to be detectable with our sampling technique is 3.15 (logarithm base-10), with 95% CL of 2.73, 3.55.

In univariate multinomial regression models, factors significantly associated with higher rectal viral load included plasma viral load ≥3.0 $\log_{10}$ copies/mL referent to undetectable plasma viral load (P < .001), lower $CD4^+$ T-cell count (P < .001), and not having been prescribed cART (P < .001) (Table 2). However, in multivariable modeling, only plasma viral load ≥3.0 $\log_{10}$ copies/mL was independently associated with higher levels of rectal viral load (odds ratio [OR] = 21.8, P = .008), although the association with prescription of cART approached significance (OR = 0.3, P = .09). $CD4^+$ T-cell count, concurrent rectal GC or CT infection, seropositivity for HSV-2, or abnormal anal cytology were not significantly associated with higher levels of rectal viral load independent of plasma viral load.

Two patterns of predicted HIV coreceptor tropism (CCR5 or CXCR4) were identified in the viral quasispecies of corresponding plasma and rectal swab samples from 4 men. For 3 men, CCR5 tropism was predicted for 59 of 60 clones from plasma and for all 60 of rectal clones (data not shown). For the fourth man, CXCR4 tropism was predicted for 18 (90%) of 20 clones from plasma but for only 12 (60%) of 20 rectal clones (P = .03). This

Table 1. Demographic and Clinical Characteristics of 80 Men Who Have Sex With Men Contributing 91 Rectal Swab Specimens as Participants in the SUN Study

Demographic or clinical characteristic	Median (25th, 75th quartiles) or percentage (no./80 men)
Race/ethnicity	
Non-Hispanic white	64 (51/80)
Non-Hispanic black	26 (21/80)
Hispanic	10 (8/80)
Median age (years)	45 (38, 50)
Clinical site	
Minneapolis	45 (36/80)
Denver	35 (28/80)
St. Louis	20 (16/80)
Median CD4 cell count (cells/μL)	467 (320, 562)
Plasma viral load (log_{10} copies/mL)	
<1.7	35 (28/80)
1.7–2.99	28 (22/80)
3.0–3.99	14 (11/80)
4.0–4.99	18 (14/80)
>5.0	6 (5/80)
Percent prescribed cART	74 (59/80)
Median duration of current cART regimen (months; n = 59)	18 (9, 37)
HSV-1 serum antibody positive	68 (53/78)
HSV-2 serum antibody positive	67 (52/78)
History of genital ulcers	5 (4/80)
Prescribed acyclovir or valacyclovir	9 (7/80)
HPV DNA positive by rectal swab	95 (76/80)
Median number of HPV serotypes detected	4 (2, 8)
Abnormal anal cytology[a]	41 (33/80)

NOTE. n = 80 unless otherwise specified; cART indicates combination antiretroviral therapy; HSV, herpes simplex virus; HPV, human papillomavirus.

[a] Atypical cells of undetermined significance, low-grade intraepithelial lesion, or high-grade intraepithelial lesion.

man had advanced HIV disease with a $CD4^+$ T-cell count of 55 cells/μL, a plasma viral load of 4.4 log_{10} copies/mL, and a rectal viral load of 5.4 log_{10} copies/swab. In addition, this man was cART-experienced and did not have rectal GC or CT infection.

DISCUSSION

Among a cohort of contemporary HIV-infected MSM receiving regular outpatient care in the United States, plasma viral load was the only independent correlate of rectal viral load in multivariable modeling that included concurrent rectal GC or CT infection, HSV-2 seropositivity, and abnormal anal cytology. In addition, concurrent GC or CT infection did not alter the fraction of rectal swabs with detectable HIV-1 RNA compared with men without rectal GC or CT infection. The majority of men in our study sample were prescribed cART and had low plasma viral loads, thus our findings cannot be readily generalized to populations where the fraction of patients with elevated plasma viral loads is greater. However, we believe our findings demonstrate that among MSM receiving contemporary antiretroviral therapy, controlling plasma viral load is an important means (in fact, perhaps the most important) of reducing rectal viral load, underscoring the value of expanded early use of cART among HIV-infected MSM in the United States to reduce HIV transmission from exposure to rectal secretions.

To our knowledge, our study is the first to use clinician-collected swabs without anoscopy to quantify HIV in rectal secretions. Previous studies have also used swabs or Sno-strips to collect rectal secretions, but all samples were collected via anoscopy [16–19]. Not requiring anoscopy could substantially ease the collection of rectal secretions for HIV quantification both for providers and for patients. No published studies have compared the various collection techniques; thus, future research in this field is warranted to validate our results.

Our data add substantially to the few published studies of HIV shedding in rectal secretions by MSM. We were able to quantify HIV RNA in rectal secretions, demonstrate the linear correlation between increasing plasma viral load and rectal viral load, and determine a threshold plasma viral load value (3.15 log_{10} copies/mL) that distinguished detectable from undetectable rectal viral load. The earliest studies associated high plasma viral load (ie, >10 000 copies/mL) with qualitative detection of HIV RNA in anal-rectal swabs but were discordant as to the effect of anal-rectal inflammation [16, 17]. More recent studies have quantified HIV in rectal secretions, but these studies did not examine some factors that could affect rectal shedding, such as concurrent GC or CT infection, HPV infection, or abnormal anal cytology [18, 19].

The most salient finding of our study was that GC or CT infection did not significantly alter the likelihood of detecting HIV in rectal secretions in our sample of MSM, most of whom were prescribed cART. Our additional finding of a stronger correlation between rectal viral load and plasma viral load for MSM with rectal GC or CT infection suggests that in the presence of ongoing viral replication, rectal virus shedding may be enhanced by co-STIs; however, we were underpowered to stratify the analysis by use of cART. A recent meta-analysis of 39 studies assessing male and female genital tract shedding in the presence of genital tract infections concluded that the odds of detecting HIV in the genital tract were significantly higher in the presence of either GC or CT [24]. In addition, HSV-2 shedding has been shown to increase the concentration of HIV in the genital tract, and treatment with valacyclovir has been shown to reduce HIV viral load in rectal secretions [18, 24]. The vast majority of these studies included subjects with high plasma viral loads who were not receiving cART. In contrast, 1 study has shown that HIV levels in semen of men with undetectable plasma viral load were not elevated in the setting of GC or CT urethritis [25]. This finding, together with our data, suggests

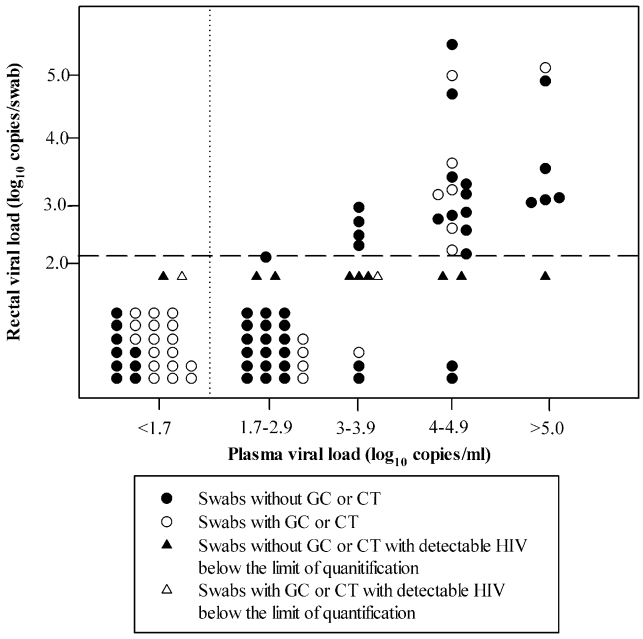

Figure 1. Correlation between plasma viral load (log_{10} copies/mL) and rectal viral load (log_{10} copies/swab) for 91 rectal swabs from 80 men who have sex with men. The horizontal dashed line is the limit of quantification for rectal swabs (2.18 log_{10} copies/swab). The vertical dotted line is the limit of quantification and detection for plasma (1.7 log_{10} copies/mL). Kendall's tau correlation coefficient for swabs without STI = 0.66 (95% CL 0.55, 0.78); for swabs with STI = 0.73 (95% CL 0.53, 0.92).

that cART may mitigate the effect of STIs on HIV transmission from infected MSM to their uninfected partners. Since recurrent rectal GC and CT infection has been associated with increased risk of HIV seroconversion in HIV-negative MSM [26], further research is needed to assess the true magnitude of rectal STIs' effect on rectal viral load and HIV transmission risk at the population level.

Currently, there is some debate regarding the use of antiretroviral therapy as a public health intervention to reduce HIV transmission [27, 28]. It is generally agreed that plasma viral load is a proxy for the infectiousness of genital tract and rectal secretions [29]. However, there is concern that transmission might still be possible in the setting of an undetectable plasma viral load, as HIV has been detected in the genital tract secretions of men and women with undetectable plasma viral loads [30, 31]. Our results are corroborated by previous findings that report rectal shedding of HIV RNA in the setting of undetectable plasma viral load is rare [17] and supports the use of cART to reduce forward transmission of HIV from rectal secretions among MSM in the United States resulting from penile exposure to rectal secretions, particularly because we saw no effect of rectal GC or CT infection on HIV detection at low plasma viral loads (<3.0 log_{10} copies/mL). In addition, this data suggest that cART will have a similar effect on reducing HIV transmission in MSM, as seen in studies of heterosexual discordant couples [32].

HIV-1 utilizes the receptor CD4 as well as a coreceptor CXCR4 or CCR5 to enter target cells. Nearly all newly infected individuals have primarily CCR5-tropic viruses in the blood; however, CXCR4-tropic viruses can emerge as HIV disease progresses [9, 10]. Our understanding of coreceptor tropism and switching is incomplete, but several factors, including viral features and host expression of the coreceptor at the inoculation site, likely contribute [11, 33–35]. Therefore, it is imperative to better understand the viral milieu at anatomic sites where HIV is likely transmitted from infected individuals, such as the rectum. Previous studies have sequenced portions of the HIV genome from rectal biopsies and feces to examine differences in HIV envelope gene (*env*) and drug-resistance profiles [12–15]. Our study is the first to examine predicted coreceptor tropism in rectal secretions. We found concordance of CCR5 tropism between plasma and rectal secretions in 3 of 4 men but significant

Table 2. Multinomial Regression Analysis With Robust Variances to Assess Factors Associated With Increased Levels of HIV Viral Load in Rectal Secretions Among 80 Men Who Have Sex With Men

	Single variable		Multivariable	
Clinical characteristic	Proportional OR	*P* value	Proportional OR	*P* value
Plasma viral load category (log_{10} copies/mL)				
≥5.0	827.7	<0.001	555.1	<0.001
4.0–4.99	268.9	<0.001	156.0	<0.001
3.0–3.99	27.3	<0.001	21.8	0.008
1.7–2.99	1.8	0.55	2.5	0.42
<1.7 (referent)	…	…	…	…
$CD4^+$ T-cell count (cells/μL, square root)	0.8	<0.001	0.9	0.12
Prescribed cART	0.1	<0.001	0.3	0.09
Rectal GC or CT infection	0.5	0.13	1.8	0.39
HSV-2 seropositive	1.2	0.74	0.6	0.46
Abnormal anal cytology	1.1	0.79	1.1	0.94

NOTE. OR, odds ratio; cART, combination antiretroviral therapy; GC, *Neisseria gonorrhoeae*; CT, *Chlamydia trachomatis*; HSV-2, herpes simplex virus-2.

discordance in a fourth man. Our preliminary findings call for further studies to delineate the extent of CCR5 and CXCR4 virus in rectal and genital secretions to better understand the molecular biology of HIV transmission from rectal secretions.

Our study has a number of limitations. First, the samples were assayed retrospectively for HIV RNA and were not collected or stored with methods optimal for the detection and quantification of RNA. However, we were able to demonstrate a strong positive correlation between plasma viral load and rectal viral load, as has been reported by other studies examining HIV concentrations in the male and female genital tract and rectal secretions [16, 17, 19, 36, 37]. Although we made extensive efforts to remove cellular material in our extraction procedures, we did detect small amounts of HIV DNA in some samples. The contaminating proviral DNA was determined to be >10- to 100-fold lower in concentration than HIV RNA; thus, our rectal viral loads and sequencing results were unlikely to be affected. Third, we were unable to test our swabs for HSV DNA shedding and instead used HSV antibody status as a correlate of chronic HSV infection. MSM who were actively shedding HSV at the time of swab collection might also have shed higher amounts of HIV and possibly confounded the association we report between plasma viral load and rectal viral load and the role of concurrent GC or CT infection. Our results may not apply to men with symptomatic proctitis. However, the majority of MSM with rectal GC or CT infections are asymptomatic [7], so our results are likely applicable in most instances.

In conclusion, our findings indicate that a low plasma HIV viral load is associated with low HIV viral load in rectal secretions. In addition, among MSM with low plasma viral load, the presence of rectal GC or CT infection does not appear to increase HIV shedding in rectal secretions. These findings support the use of cART as an effective means of reducing HIV transmission among MSM in the United States by reducing the amount of virus shed in body sites where transmission occurs.

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References

1. Trends in HIV/AIDS diagnoses among men who have sex with men—33 states, 2001–2006. MMWR Morb Mortal Wkly Rep **2008**; 57:681–6.
2. Centers for Disease Control and Prevention. HIV Surveillance Report, **2008**; 20. http://www.cdc.gov/hiv/topics/surveillance/resources/reports/. Accessed 1 December 1.
3. Jaffe HW, Valdiserri RO, De Cock KM. The reemerging HIV/AIDS epidemic in men who have sex with men. JAMA **2007**; 298:2412–4.
4. Hall HI, Song R, Rhodes P, et al. Estimation of HIV incidence in the United States. JAMA **2008**; 300:520–9.
5. Sullivan PS, Salazar L, Buchbinder S, Sanchez TH. Estimating the proportion of HIV transmissions from main sex partners among men who have sex with men in five US cities. AIDS **2009**; 23:1153–62.
6. Cohen MS, Hoffman IF, Royce RA, et al. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet **1997**; 349:1868–73.
7. Kent CK, Chaw JK, Wong W, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. Clin Infect Dis **2005**; 41:67–74.
8. Scott KC, Philip S, Ahrens K, Kent CK, Klausner JD. High prevalence of gonococcal and chlamydial infection in men who have sex with men with newly diagnosed HIV infection: an opportunity for same-day presumptive treatment. J Acquir Immune Defic Syndr **2008**; 48:109–12.
9. van't Wout AB, Kootstra NA, Mulder-Kampinga GA, et al. Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. J Clin Invest **1994**; 94:2060–7.
10. Zhu T, Mo H, Wang N, et al. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science **1993**; 261:1179–81.
11. Moore JP, Kitchen SG, Pugach P, Zack JA. The CCR5 and CXCR4 coreceptors—central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses **2004**; 20:111–26.
12. van der Hoek L, Sol CJ, Snijders F, Bartelsman JF, Boom R, Goudsmit J. Human immunodeficiency virus type 1 RNA populations in faeces with higher homology to intestinal populations than to blood populations. J Gen Virol **1996**; 77:2415–25.
13. van Marle G, Gill MJ, Kolodka D, McManus L, Grant T, Church DL. Compartmentalization of the gut viral reservoir in HIV-1 infected patients. Retrovirology **2007**; 4:87.
14. Poles MA, Elliott J, Vingerhoets J, et al. Despite high concordance, distinct mutational and phenotypic drug resistance profiles in human immunodeficiency virus type 1 RNA are observed in gastrointestinal mucosal biopsy specimens and peripheral blood mononuclear cells compared with plasma. J Infect Dis **2001**; 183:143–8.
15. Monno L, Punzi G, Scarabaggio T, et al. Mutational patterns of paired blood and rectal biopsies in HIV-infected patients on HAART. J Med Virol **2003**; 70:1–9.
16. Kiviat NB, Critchlow CW, Hawes SE, et al. Determinants of human immunodeficiency virus DNA and RNA shedding in the anal-rectal canal of homosexual men. J Infect Dis **1998**; 177:571–8.
17. Lampinen TM, Critchlow CW, Kuypers JM, et al. Association of antiretroviral therapy with detection of HIV-1 RNA and DNA in the anorectal mucosa of homosexual men. AIDS **2000**; 14:F69–75.
18. Zuckerman RA, Lucchetti A, Whittington WL, et al. Herpes simplex virus (HSV) suppression with valacyclovir reduces rectal and blood plasma HIV-1 levels in HIV-1/HSV-2-seropositive men: a randomized, double-blind, placebo-controlled crossover trial. J Infect Dis **2007**; 196:1500–8.
19. Zuckerman RA, Whittington WL, Celum CL, et al. Higher concentration of HIV RNA in rectal mucosa secretions than in blood and seminal plasma, among men who have sex with men, independent of antiretroviral therapy. J Infect Dis **2004**; 190:156–61.
20. Vellozzi C, Brooks JT, Bush TJ, et al. The study to understand the natural history of HIV and AIDS in the era of effective therapy (SUN Study). Am J Epidemiol **2009**; 169:642–52.
21. Sullivan ST, Mandava U, Evans-Strickfaden T, Lennox JL, Ellerbrock TV, Hart CE. Diversity, divergence, and evolution of cell-free human

immunodeficiency virus type 1 in vaginal secretions and blood of chronically infected women: associations with immune status. J Virol **2005**; 79:9799–809.
22. Chen J, Young NL, Subbarao S, et al. HIV type 1 subtypes in Guangxi Province, China, 1996. AIDS Res Hum Retrov **1999**; 15:81–4.
23. MCCullagh P. Regression models for ordinal data (with discussion). J R Statist Soc Ser B **1980**; 42:109–42.
24. Johnson LF, Lewis DA. The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. Sex Transm Dis **2008**; 35:946–59.
25. Sadiq ST, Taylor S, Kaye S, et al. The effects of antiretroviral therapy on HIV-1 RNA loads in seminal plasma in HIV-positive patients with and without urethritis. AIDS **2002**; 16:219–25.
26. Bernstein KT, Marcus JL, Nieri G, Philip SS, Klausner JD. Rectal gonorrhea and chlamydia reinfection is associated with increased risk of HIV seroconversion. J Acquir Immune Defic Syndr **2010**; 53:537–43.
27. Granich RM, Gilks CF, Dye C, De Cock KM, Williams BG. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. Lancet **2009**; 373:48–57.
28. Dodd PJ, Garnett GP, Hallett TB. Examining the promise of HIV elimination by 'test and treat' in hyperendemic settings. AIDS **2010**; 24:729–35.
29. Cohen MS, Gay C, Kashuba AD, Blower S, Paxton L. Narrative review: antiretroviral therapy to prevent the sexual transmission of HIV-1. Ann Intern Med **2007**; 146:591–601.
30. Neely MN, Benning L, Xu J, et al. Cervical shedding of HIV-1 RNA among women with low levels of viremia while receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr **2007**; 44: 38–42.
31. Sheth PM, Kovacs C, Kemal KS, et al. Persistent HIV RNA shedding in semen despite effective antiretroviral therapy. AIDS **2009**; 23: 2050–4.
32. Attia S, Egger M, Muller M, Zwahlen M, Low N. Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. AIDS **2009**; 23:1397–404.
33. Yeaman GR, Asin S, Weldon S, et al. Chemokine receptor expression in the human ectocervix: implications for infection by the human immunodeficiency virus-type I. Immunology **2004**; 113:524–33.
34. Hladik F, Lentz G, Delpit E, McElroy A, McElrath MJ. Coexpression of CCR5 and IL-2 in human genital but not blood T cells: implications for the ontogeny of the CCR5+ Th1 phenotype. J Immunol **1999**; 163: 2306–13.
35. Patterson BK, Landay A, Andersson J, et al. Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. Am J Pathol **1998**; 153:481–90.
36. Barroso PF, Schechter M, Gupta P, et al. Effect of antiretroviral therapy on HIV shedding in semen. Ann Intern Med **2000**; 133:280–4.
37. Hart CE, Lennox JL, Pratt-Palmore M, et al. Correlation of human immunodeficiency virus type 1 RNA levels in blood and the female genital tract. J Infect Dis **1999**; 179:871–82.